

Asbestos bodies in bronchoalveolar lavage fluids of brake lining and asbestos cement workers

P Dumortier, P De Vuyst, P Strauss, J C Yernault

Abstract

Asbestos body (AB) concentrations in bronchoalveolar lavage samples of 15 brake lining (BL) workers exposed only to chrysotile have been determined and compared with those from 44 asbestos cement (AC) workers extensively exposed to amphiboles. The mean AB concentrations (263 ± 802 and 842 ± 2086 AB/ml respectively) for those groups did not differ significantly but were much higher than those found in control groups. Analytical electron microscopy of asbestos body cores showed that in the BL group 95.6% were chrysotile fibres whereas in the AC group amphiboles accounted for 93.1%. The size characteristics of the central fibres differed for chrysotile and amphibole AB, the former being shorter and thinner. Examination of repeated bronchoalveolar lavage samples showed that the mechanisms of clearance of chrysotile fibres do not affect AB concentration for at least 10 months after cessation of exposure. It thus appears that routine counting of ABs in BAL allows the assessment of current or recent occupational exposures to asbestos. Exposures to chrysotile lead to AB concentrations comparable with those encountered in exposures to amphiboles.

Treatment of bronchoalveolar lavage (BAL) fluids by digestion of the organic material and collection on membrane filters allows the recovery of samples suitable for light and electron microscopy counting and analysis of mineral particles. Several studies have pointed out the usefulness of detecting asbestos bodies (ABs)^{1,2} and fibres³ in BAL fluids. Indeed, light microscopy counting of ABs is an easy technique for detecting an important portion of the

longest asbestos fibres present in the BAL samples. It has become a routine technique in some laboratories and has proved a useful aid in evaluating occupational⁴ and environmental⁵ exposures to asbestos. Moreover, concentrations of ABs in BAL fluids may be used to evaluate roughly the lung tissue content of ABs as shown by two recent independent studies.^{6,7} Both studies derived similar relations between the asbestos body content of BAL fluids and tissue samples.

Dimensional characteristics of the fibres involved in AB formation show that those smaller than $10 \mu\text{m}$ rarely become coated⁸⁻¹¹ and that the probability of coating increases with fibre length.⁹ The longest AB reported reached $360 \mu\text{m}$.⁹ The mean diameter of AB central fibres is essentially dependent on the nature of the fibre involved.^{8,10}

In man the relative proportions of coated and uncoated fibres and the coating rate varies between individuals.⁹ In rodents Davis has shown experimentally that AB formation takes less than two months; however, this reaction does not affect all the fibres at the same time.¹²

Chrysotile accounts for more than 90% of the world's asbestos consumption whereas of the amphiboles, only crocidolite and amosite are of major economic importance. Anthophyllite, tremolite, and actinolite are usual contaminants of chrysotile, talc, and vermiculite ores.¹³ Reported electron microscope analyses of AB cores show that many ABs are built on amphibole fibres. This raises questions as to the validity of ABs as markers of exposure to chrysotile. Although chrysotile was reported to occur in significant proportions (64%) of the AB cores analysed in tissue samples of chrysotile miners¹⁴ and to form in animal experiments,¹⁵ it is currently thought that ABs form rather poorly on chrysotile fibres compared with amphiboles, even in exposed subjects.⁸ Despite its widespread use chrysotile is only occasionally found in the form of ABs in several cohorts.¹⁶⁻¹⁹

We report in this paper a comparative study of the concentrations and the nature of ABs encountered in the BAL of brake lining factory workers exposed only to chrysotile and those of asbestos cement workers exposed extensively to amphiboles. The nature and dimensional characteristics of AB cores in these

Research Unit on the Toxicity of Mineral Particles, Chest Department, Erasme University Hospital, Brussels

P Dumortier, P De Vuyst, J C Yernault
Occupational Disease Fund, Brussels, Belgium
P Strauss

groups were assessed by analytical electron microscopy. Pathological characteristics of both groups are also discussed.

Material and methods

SUBJECTS SELECTION AND DATA

As of the present, our laboratory has received more than 1500 BAL samples for routine counting of ABs. Among these samples we recorded 15 subjects all employed in the same brake lining factory (BL group) and 44 in two different asbestos cement plants (AC group). Among the subjects in the AC group, 32 were working in plant 1 and 12 in plant 2. The BL factory has used only chrysotile imported from Canada and Italy for the whole of its history (20 years). AC plant 1 used mainly crocidolite until 1981 when it was totally replaced by chrysotile and AC plant 2 used crocidolite and amosite until 1983 when they became totally replaced by chrysotile and non-asbestos fibres.

Data for each subject (age, duration of exposure, time since end of exposure, chest x ray image, and histological confirmation of mesothelioma or bronchial carcinoma) were collected either from the referring physician or, when available, from records of the Occupational Disease Fund. These data were available for all the subjects of the BL group and for 37 of the AC group, only partial data being obtained for the seven remaining subjects.

All were men. The mean durations of exposure are reported in table 1 and clinical data for each group in table 2. All subjects with bronchial carcinoma were smokers. Repeated BAL samples were obtained in three subjects of the BL group. Delays between repeated BAL samples expressed in months were 18, one, and seven, and between end of exposure and the second BAL sample 10, zero, and one respectively.

SAMPLE PREPARATION AND ASBESTOS BODY COUNTING

Methods of sampling, processing, and light microscopy counting have been described in detail elsewhere.² Briefly, 10–30 ml of BAL fluid are treated with sodium hypochloride (commercial bleach) to destroy organic material (cells, mucus). The remain-

Table 2 Clinical data

	Brake lining (n = 15)	Asbestos cement (n = 38)†
Radiographic evidence of asbestos related diseases*	—	24
Mesothelioma	—	6
Bronchial carcinoma	2	4
No specific radiographic changes and no respiratory malignancy	13	7

*Asbestosis, pleural or diaphragmatic plaques, pleural thickening.

†Clinical data were not available for six subjects of this group of 44.

ing inorganic particles are collected by filtration on Millipore membrane filters (0.45 µm nominal porosity). The filters are clarified and fixed on glass slides with acetone vapours. ABs are routinely counted under a phase contrast light microscope at 250 ×. Only ferruginous bodies closely corresponding to the definition of typical asbestos bodies¹⁰ were taken into account in further calculations. Concentrations were expressed in numbers of asbestos bodies per ml of BAL fluid.

SAMPLES SELECTION AND PREPARATION FOR ELECTRON MICROSCOPY

In each group the samples with the highest AB concentrations were selected for analytical electron microscopy. Twenty five light microscopy slides, seven from the BL group, 13 from AC plant 1, and five from AC plant 2 were retained. The filters were uncovered and the immersion oil removed with carbon tetrachloride. Small pieces of filter were cut out and carbon coated. The filter material was then dissolved with acetone in a modified Jaffe washer, leaving the particles embedded in the carbon film on an electron microscope grid. "Slim bar" 300 mesh copper grids (SPI cat No 2130C) with grid bars 10 µm wide and 77% open area were used to facilitate the detection and sizing of ABs. For samples with a calculated mean AB concentration higher than 10 AB per square millimetre of filter, areas were randomly selected and directly examined by electron microscopy. For concentrations between 2 and 10 AB/mm², asbestos bodies must first be located by light microscopy inspection of the grids and their positions noted on low magnification (25 ×) polaroid photographs. For concentrations lower than 2 AB/mm², high precision object marking is necessary before filter cutting.²⁰

ANALYTICAL ELECTRON MICROSCOPY

The AB central fibres are analysed and sized with a Philips EM400T transmission electron microscope fitted with a scanning attachment and an EDAX PV 9900 x ray energy dispersive spectrometer. The transmission image is inspected at a magnification of 8000 × to detect AB. Length is measured at the same

Table 1 Summary of subject data

	Brake lining (n = 15)	Asbestos cement (n = 44)	p Value
Mean age (y)	45.5 ± 9.0	53.4 ± 10.5	< 0.01
Mean duration of exposure (y)	14.5 ± 2.5	18.9 ± 9.5	NS
Range	10–17	0.5–35	
Delay since end of exposure (months)	0.9 ± 2.4	66 ± 1108	< 0.005
Range (y)	0–0.8	0–45	
Mean AB concentration (AB/ml of BAL)	263 ± 802	842 ± 2086	NS
Range	0.2–3168	0.3–11200	

magnification. Secondary electron imaging is used when ABs partially lie on a grid bar. Evaluation of the morphological characters and diameter sizing are performed at magnifications between 22 000 and 60 000 \times . When possible, the area of the central fibre without iron protein coating or only slightly coated are selected for chemical analysis. Spectra obtained from UICC standard and a tremolite asbestos sample are used as references. Identification of the asbestos type is made by integrating all the information available from morphological observations, electron diffraction patterns, and chemical analysis. It is relatively straightforward to identify chrysotile, anthophyllite and tremolite on this basis. In most cases it is also possible to distinguish between the cores of amosite and crocidolite asbestos bodies by comparing their spectra with reference spectra. In cases of doubt, however, fibres are classified as "unresolved commercial amphibole." When chemical spectra and electron diffraction patterns do not match reference spectra, they are classified as non-asbestos and when data are insufficient to identify the type of fibre, mainly because the iron protein coating is too thick, they are classified as undetermined.

STATISTICAL ANALYSIS

Unless otherwise stated, for each analysed variable, the arithmetic mean followed by the standard error is

presented in the results. The *t* test is used to evaluate statistical differences.

Results

ASBESTOS BODY COUNTING

All subjects in the BL and AC groups had ABs in their BAL fluid. Mean concentrations do not differ significantly between these two groups (table 1, fig 1) but are much higher than those encountered in previously published controls.² The proportion of subjects having more than 1 AB/ml is 86.7% for the BL group and 97.9% for the AC group. Concentrations of more than 10 AB/ml were recorded in 60% and 73.2% respectively compared with 0.8 and 0% in blue and white collar worker controls.

Values obtained for repeated BAL samples are within the reproducibility limits of the method and give respectively 273 and 351, 13.5 and 15, and 3119 and 3165 AB/ml.

Light microscopy examination of the ABs in the BL and AC groups showed a pronounced difference in their morphology, length, and coating thickness. Asbestos bodies were less coated, shorter, and less characteristic in the BL group (fig 2).

ASBESTOS BODY CENTRAL FIBRE ANALYSIS

From eight to 48 central fibres (mean: 26.0 ± 10.5) were examined by electron microscopy in each selected sample. Among the 677 analysed cores, tremolite was never found, eight (1.2%) remained undetermined, and one was a pseudoasbestos body on a silica fibre.

Chrysotile was identified in the seven samples analysed for the BL group and accounts for 95.6% of the 159 central fibres analysed (fig 3). Grids from two BL samples with 273 and 231 AB/ml were also extensively examined at 22 000 \times to detect uncovered tremolite fibres; few were found (five and three on each grid respectively). These fibres were shorter than 10 μ m and had aspect ratios lower than 20.

Among the 518 cores analysed in the AC group, amphiboles accounted for 93.1%, all but one being commercial amphiboles (amosite or crocidolite), the remaining one being an anthophyllite core. Commercial amphiboles were present in all 18 analysed subjects of this group, amosite in 10, and crocidolite in 17 of them. Chrysotile was found in 11 AC subjects. It appears from table 3 that different local situations exist between the two AC subgroups.

DIMENSIONAL CHARACTERISTICS OF ASBESTOS BODY CENTRAL FIBRES

Morphological differences between ABs on chrysotile and amphiboles are evident from fig 2. Length, diameter, and aspect ratio of central fibres are summarised in table 4. For chrysotile cores, there is no difference in size characteristics when consider-

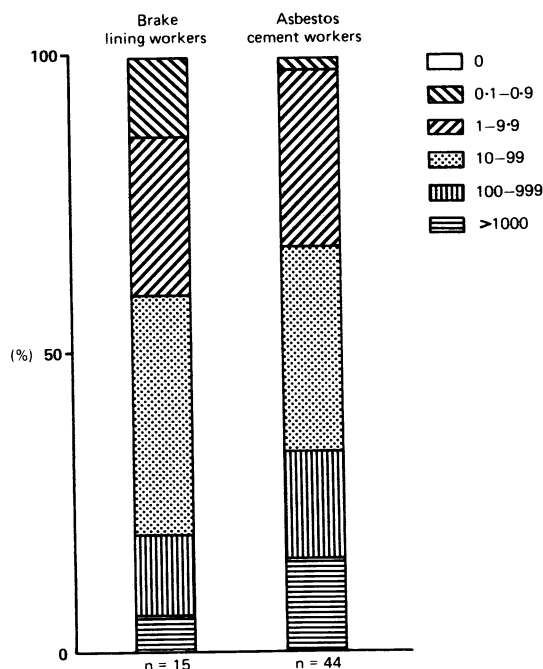


Figure 1 Asbestos body concentrations in BALs of brake lining and asbestos cement workers.

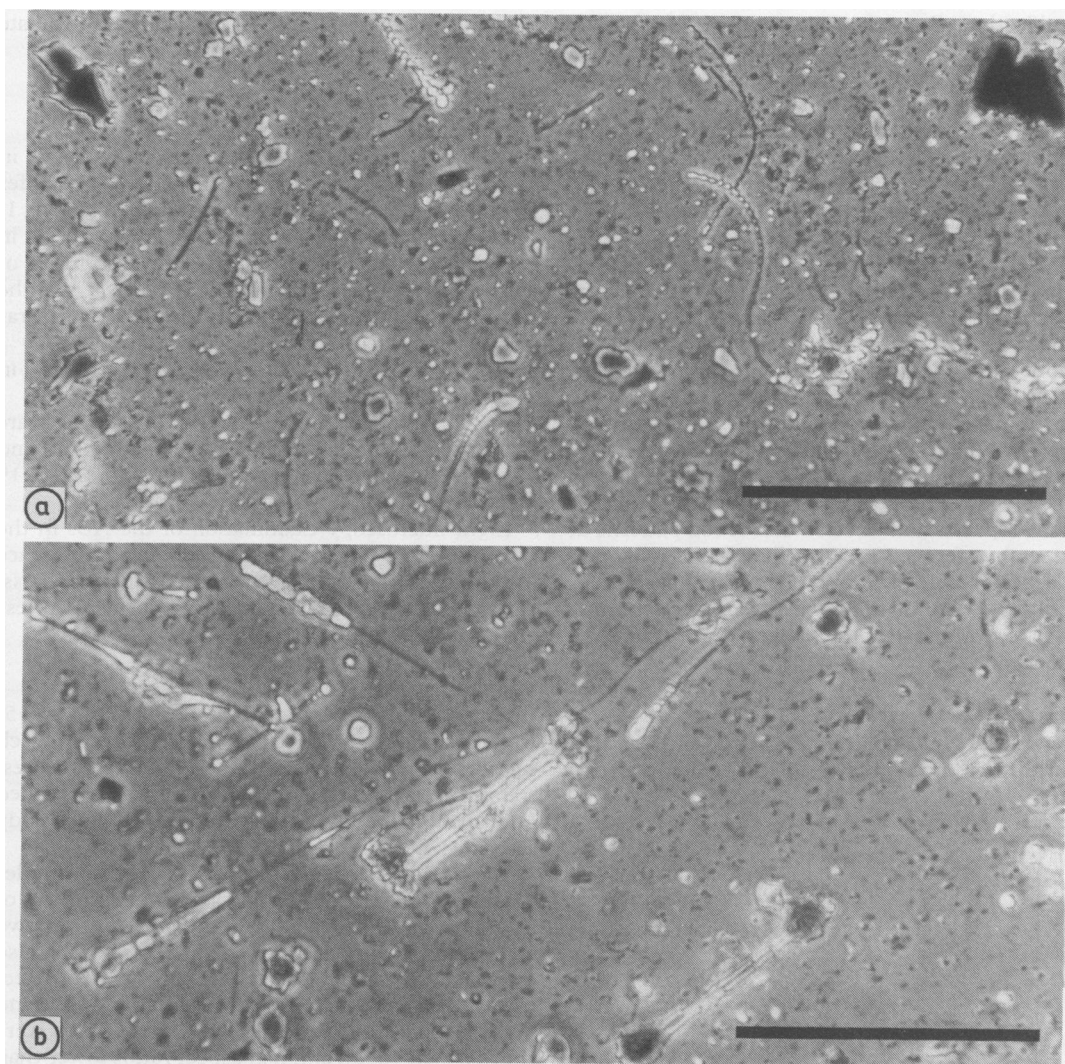


Figure 2 Phase contrast light microscopy of BAL (a) of brake lining worker with all asbestos bodies built on chrysotile fibres (513 AB/mm^2 of filter) and (b) of an asbestos cement worker with 60% of AB built on crocidolite and 40% on amosite (406 AB/mm^2 of filter). Scale bar $50 \mu\text{m}$.

ing only those from BL subjects or when pooling them all together: 19.8% of the AB on chrysotile had a single fibril as their cores (fig 4). The longest AB on a single fibril was $63 \mu\text{m}$. Several bodies were found on chrysotile fibres with multiple splitting. Although the arithmetic mean diameters of chrysotile and crocidolite are similar, the distribution of diameters differs (fig 5); the geometric mean diameters were 5.5×10^{-2} and $9.7 \times 10^{-2} \mu\text{m}$ respectively ($p < 0.01$). It must be noted that 81.5% of the chrysotile, 87.8% of the crocidolite, and 28.6% of the amosite cores are less than $0.2 \mu\text{m}$ in diameter and would therefore remain undetected by phase contrast light micros-

copy in the absence of coating. As regards the length and the aspect ratio, asbestos bodies on chrysotile are shorter ($p < 0.01$) but have a higher aspect ratio ($p < 0.01$) than those on amphiboles.

Discussion

This study compares asbestos body concentrations and central fibre types in BAL fluids from a cohort of subjects exposed only to chrysotile to another exposed extensively to amphiboles. It allows us to confirm the diagnostic value of ABs as indicators of any current or recent occupational exposure to asbestos, even to chrysotile.

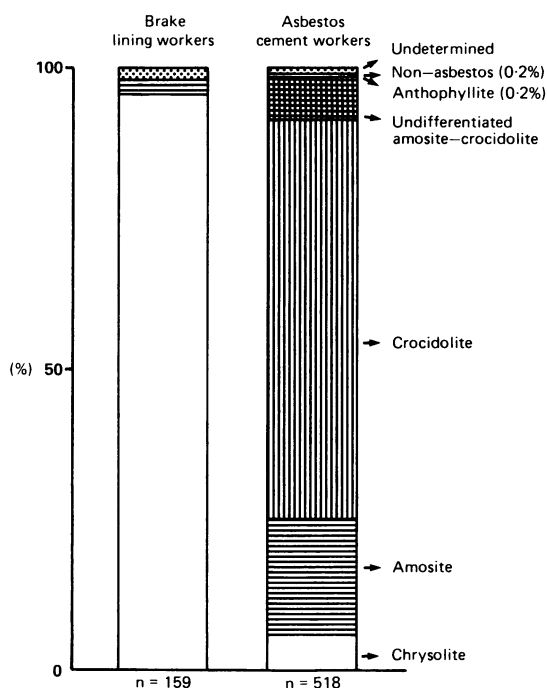


Figure 3 Asbestos body core analysis in brake lining and asbestos cement workers.

The difference in the mean length of $35 \pm 15 \mu\text{m}$ reported by Holden and Churg for chrysotile asbestos bodies in their cohort of chrysotile miners¹⁴ and the $25 \pm 15 \mu\text{m}$ value observed in the present study of subjects mainly mixing raw material, sawing, drilling, and rectifying chrysotile bearing parts may be explained in two ways. Firstly, differences probably exist in the granulometric curves of the aerosols to which the two populations were exposed. This hypothesis is supported by the observation of Gibbs and Hwang showing that significant differences occur in airborne fibre sizes between mining and bagging areas of the same asbestos mine.²¹ Moreover, uncovered fibre analysis performed on lung tissues of workers exposed to processed chrysotile has shown that pronounced differences may occur in the mean chrysotile fibre length as a function of the

Table 4 Dimensional characteristics of AB cores

	Chrysotile	Crocidolite	Amosite
Length (μm):			
Mean \pm SD	24.8 ± 14.8	34.4 ± 23.6	49.2 ± 28.5
Range	6–120	10–276	15–184
Diameter (μm):			
Mean \pm SD	0.11 ± 0.15	0.12 ± 0.09	0.27 ± 0.17
Range	0.01–0.75	0.01–0.7	0.04–1.0
Aspect ratio:			
Mean \pm SD	738 ± 814	382 ± 280	241 ± 166
Range	24–6300	46–2208	15–920

occupational category of the subjects.²² Secondly, the type of samples used in the two studies is not the same and there are actually no published data available comparing the respective sizes of asbestos bodies in BAL and tissue samples of the same subjects. For uncovered fibres it was shown that those of alveolar spaces recovered by BAL are significantly shorter than those of the lung parenchyma.²³

Because of the smaller size of ABs on chrysotile, phase contrast light microscopy and magnifications of $250\times$ or higher are advisable to perform correct AB counting in cases of suspected chrysotile exposures. On the other hand, a large proportion of all the fibres affected in AB formation have diameters smaller than $0.2 \mu\text{m}$. This implies that these fibres would not be detected during routine monitoring by phase contrast light microscopy of airborne asbestos fibres in occupational environments.

With possible restrictions due to differences in the duration and delay since the end of exposure, concentrations in the BL and AC groups are comparable. Possible reasons for the overlap of the lowest concentrations ($< 10 \text{ AB/ml}$) in these groups with those encountered in controls are: the quality of BAL² and the variation of exposure intensity in the factories. Subjects included in the studied groups held a variety of jobs such as asbestos mixing, raw material moulding, parts sawing, or drilling and storekeeping.

Concerning the persistence of ABs on chrysotile in BAL, the only objective data resulting from our study show that there is no evident change in asbestos body concentration within the first 10 months after cessation of exposure. The problem of the persistence of chrysotile ABs was not mentioned in the paper of Holden and Churg.¹⁴ It is important, however, to know if such bodies are subject to extensive clearance as are uncovered chrysotile fibres.^{24,25} This contrasts with the low clearance rate of amphibole ABs which may remain in the lungs tens of years after exposure ends.⁵ The observation that asbestos bodies form poorly on chrysotile fibres may result from a differential clearance of chrysotile and amphibole asbestos bodies.^{8,11} Effectively, in this hypothesis chrysotile and amphibole asbestos bodies would be formed in similar proportions, but if a sufficiently long time has elapsed since the end of

Table 3 Asbestos body core analysis in the AC group*

Central fibre type	AC plant 1 (n = 405)	AC plant 2 (n = 113)
Chrysotile	5.9%	5.3%
Crocidolite	74.3%	38.1%
Amosite	10.9%	49.6%
Total commercial amphiboles†	93.1%	92.0%
Anthophyllite	—	0.9%

*Undetermined and non-asbestos body cores not reported here.

†Crocidolite + amosite + unresolved amosite/crocidolite (see text).

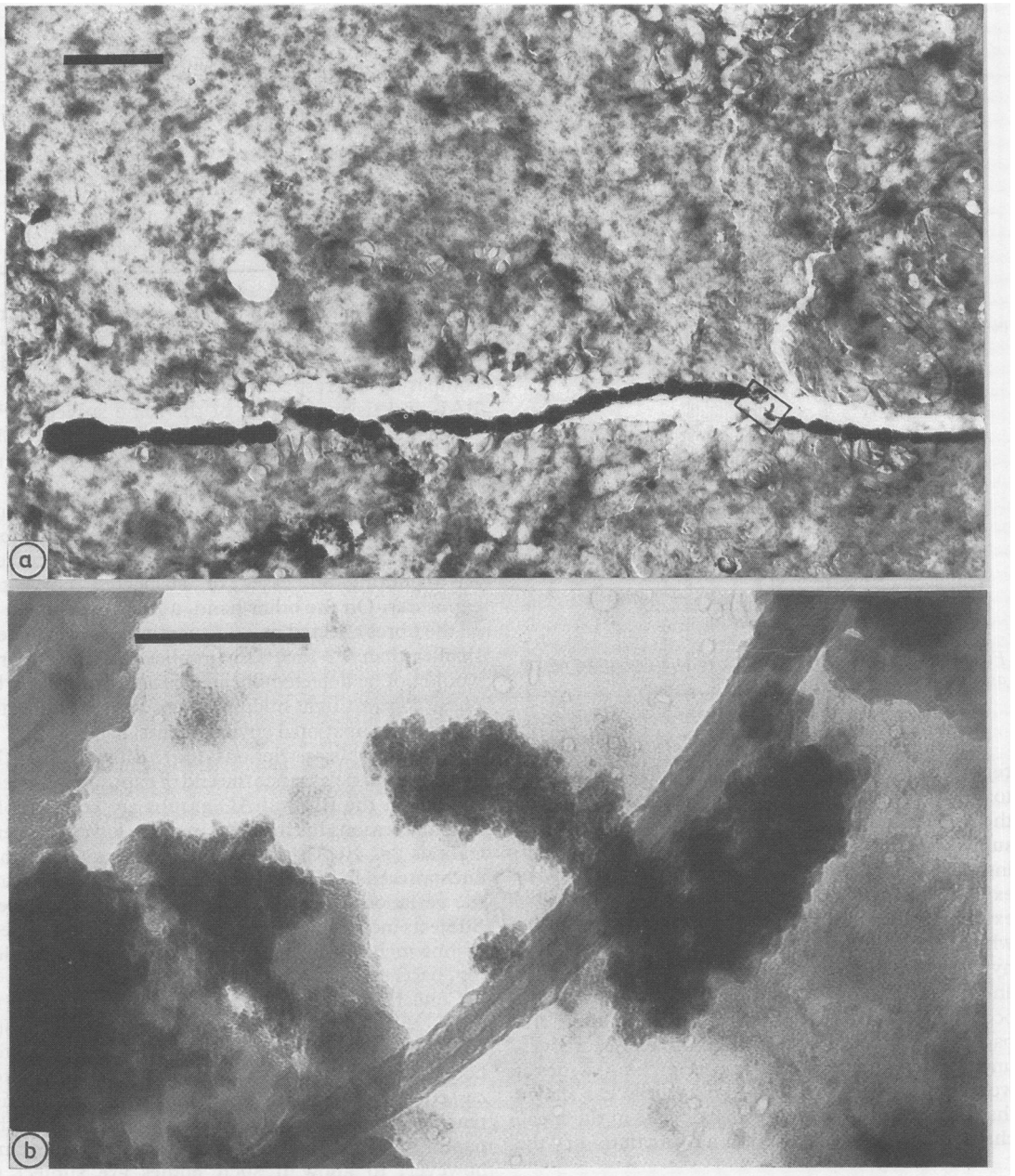


Figure 4 Asbestos body on a long single fibril of chrysotile. Total length of asbestos body is 52 μm . (a) General view, scale bar 2 μm . (b) Detail of single fibril protruding from ferroprotein coating, scale bar 0.1 μm .

exposure at the moment lung samples are analysed most of the chrysotile ABs would be cleared whereas those on the amphiboles would still be present. Possible causes for a better clearance of chrysotile ABs include: better penetration and transport in the tissue due to their smaller size and the probability

that the chrysotile fibres remain subject to increased segmentation and defibrillation even in a coated form. It is thus clear that more experimental data are required to evaluate the persistence of chrysotile ABs in BAL and tissue samples.

Repeated BAL samples on animals¹⁵ or on volun-

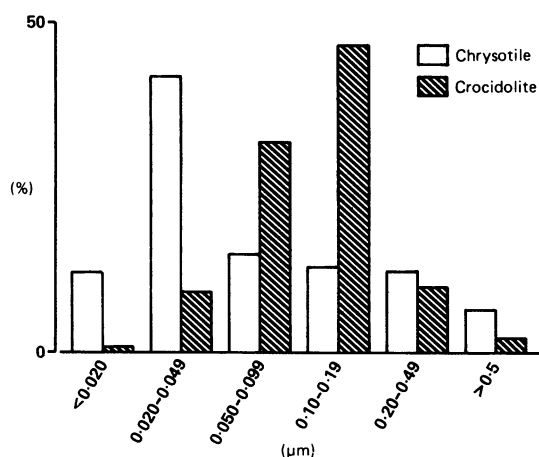


Figure 5 Granulometric curve of chrysotile and crocidolite asbestos body central fibre diameters.

teers with pure chrysotile exposures would allow the determination of the length of time chrysotile ABs may be found after the end of exposure. This would help to assess the value of ABs in BAL as indicators of occupational exposure to chrysotile.

Apart from clearance mechanisms, another reason may be invoked for the possible absence of AB in histological sections of chrysotile workers as suggested by Becklake.²⁶ Owing to their smaller size and less characteristic shape, AB on chrysotile could be more easily overlooked than those on amphiboles, especially in sections.

Whether the long single fibrils of chrysotile forming the cores of ABs are inhaled in this form or result from the defibrillation of thicker fibres in the lung is not clear. Nevertheless, the thinness of the fibres is not a limiting factor with respect to coating, unlike their length. This implies that long single fibrils, as small as 0.01 µm in diameter, may induce some biological reaction.

Despite the fact that few uncovered tremolite fibres with low aspect ratios were detected in subjects of the BL group, the absence of ABs on contaminant amphiboles (anthophyllite or tremolite) suggest that few, if any, long fibres of these types are present in the subjects. This thus indicates an almost "pure" exposure to chrysotile in the brake lining factory. The follow up of such a cohort may be used to evaluate pathological changes due to pure chrysotile inhalation in man.

It appears from this study that the asbestos cement industry cannot be considered as a whole with regard to the type of fibre used since it varies from plant to plant and even from division to division within the same plant and since it is subject to changes with time and evolution of the technology. Thus one must be careful when comparing data about cohorts of AC

workers and take the type of fibres to which they were exposed into consideration. Although chrysotile has been the only fibre used in AC plant 1 since 1981 and in AC plant 2 since 1983, occurrence of chrysotile ABs in AC subjects is relatively low when compared with those on amphiboles. This may result from a differential clearance as evoked earlier but the conjunction of this factor with the progressive improvement in the working conditions must also be taken into account. The consequence of this improvement is a continuous reduction of the maximum airborne fibre level in the environment. This implies that the subjects were exposed to higher concentrations of amphiboles in the past than of chrysotile today. It must also be noted that some AC subjects stopped working before the use of amphiboles ceased and that they were therefore less subject to chrysotile exposure.

Concerning the apparent differences in diseases observed in BL and AC groups, several biasing factors must be considered besides the differences in fibre type—namely, age, duration of exposure, and period since the end of exposure. Nevertheless, the absence of non-malignant asbestos related diseases and of mesothelioma in the BL group, which is confirmed to a larger extent for the whole factory with data furnished by the Occupational Disease Fund, must be taken into consideration. This may be related to a lesser toxic effect of exposures to chrysotile.

We thank Mrs B Mahaux for typing this manuscript and Mrs A Howard for reviewing it.

- 1 Sebastien P. Possibilités actuelles de la biométrie des poussières sur échantillon de liquide de lavage broncho-alvéolaire. *Ann Biol Clin* 1982;40:279-93.
- 2 De Vuyst P, Dumortier P, Moulin E, Yourassowsky N, Yernault JC. Diagnostic value of asbestos bodies in bronchoalveolar lavage fluid. *Am Rev Respir Dis* 1987;136:1219-24.
- 3 Gellert AR, Kitajewska JY, Uthayakumars S, Kirkham JB, Rudd RM. Asbestos fibers in bronchoalveolar lavage fluid from asbestos workers: examination by electron microscopy. *Br J Ind Med* 1986;43:170-6.
- 4 Di Menza I, Hirsch A, Sebastien P, Gaudichet A, Bignon J. Assessment of past asbestos exposure in patients: occupational questionnaire versus monitoring in broncho-alveolar lavage. In: Wagner JC, ed. *Biological effects of mineral fibres*. Lyon: International Agency for Research on Cancer, 1980:609-14. (Monograph 30.)
- 5 De Vuyst P, Mairesse M, Gaudichet A, Dumortier P, Jedwab J, Yernault JC. Mineralogical analysis of bronchoalveolar fluid as an aid to diagnosis of "imported" pleural asbestosis. *Thorax* 1983;38:628-9.
- 6 Sebastien P, Armstrong B, Monchaux G, Bignon J. Asbestos bodies in bronchoalveolar lavage fluid and lung parenchyma. *Am Rev Respir Dis* 1988;137:75-8.
- 7 De Vuyst P, Dumortier P, Moulin E, et al. Asbestos bodies in bronchoalveolar lavage reflect lung asbestos body concentration. *Eur Respir J* 1988;1:362-7.
- 8 Pooley FD. Asbestos bodies, their formation, composition and characters. *Environ Res* 1972;5:363-79.
- 9 Morgan A, Holmes A. Concentrations and dimensions of coated and uncoated asbestos fibres in human lung. *Br J Ind Med* 1980;37:25-32.

- 10 Churg A, Warnock ML. Asbestos and other feruginous bodies: their formation and clinical significance. *Am J Pathol* 1981;102:447-56.
- 11 Morgan A, Holmes A. The enigmatic asbestos body: its formation and significance in asbestos-related disease. *Environ Res* 1985;38:283-92.
- 12 Davis JMG. The ultrastructure of asbestos bodies from guinea-pig lungs. *Br J Exp Pathol* 1964;45:642-6.
- 13 Wagner JC. Mineral fibres and mesothelioma. *Thorax* 1986;41:161-6.
- 14 Holden J, Churg A. Asbestos bodies and the diagnosis of asbestosis in chrysotile workers. *Environ Res* 1986;39:232-6.
- 15 Begin R, Masse S, Sebastien P, et al. Asbestos exposure and retention as determinants of airway disease and asbestos alveolitis. *Am Rev Respir Dis* 1986;134:1176-81.
- 16 Churg A, Warnock ML. Analysis of the cores of ferruginous (asbestos) bodies from the general population. I. Patients with and without lung cancer. *Lab Invest* 1977;37:280-6.
- 17 Churg A, Warnock ML. Analysis of the cores of ferruginous (asbestos) bodies from the general population. III. Patients with environmental exposure. *Lab Invest* 1979;40:622-6.
- 18 Roggli VL, Pratt PC, Brody AR. Asbestos content of lung tissue in asbestos associated diseases: a study of 110 cases. *Br J Ind Med* 1986;43:18-28.
- 19 Roggli VL, McGavran MH, Subach J, Sybers HD, Greenberg SD. Pulmonary asbestos body counts and electron probe analysis of asbestos body cores in patients with mesothelioma: a study of 25 cases. *Cancer* 1982;50:2423-32.
- 20 Dumortier P, De Vuyst P. Object marking, a bridge between light and analytical electron microscopy for particles characterization. *Journal of Electron Microscopy Techniques* 1988;8:229-30.
- 21 Gibbs GW, Hwang CY. Dimensions of airborne asbestos fibers. In: Wagner JC, ed. *Biological effects of mineral fibers*. Lyon: International Agency for Research on Cancer, 1980:69-78. (IARC monograph 30.)
- 22 Bignon J, Sebastien P, Gaudichet A. Measurement of asbestos retention in the human respiratory system related to health effects. In: Gravatt CC, Lafleur PD, Heinrich KFJ, eds. *Workshop on asbestos: definition and measurement methods*. Washington: National Bureau of Standards, 1978:95-119. (NBS spec publ 506.)
- 23 Churg A, Wiggs B. Fiber size and number in workers exposed to processed chrysotile asbestos, chrysotile miners, and the general population. *Am J Ind Med* 1986;9:143-52.
- 24 Wagner JC, Berry G, Skidmore JW, Timbrell V. The effects of inhalation of asbestos in rats. *Br J Cancer* 1974;29:252-69.
- 25 Gylseth B, Mowe G, Wannag A. Fibre type and concentration in the lungs of workers in an asbestos cement factory. *Br J Ind Med* 1983;40:375-9.
- 26 Becklake MR. Asbestosis criteria. *Arch Pathol Lab Med* 1984;108:93.

Accepted 13 March 1989